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Research Article

Acute Toxicity and Cytogenetic Effects of Monocrotophos in *Paramecium Caudatum* and *Oxytricha Fallax*

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ABSTRACT

In the present study experiments were conducted to evaluate the toxic effects of monocrotophos on ciliate models *Paramecium caudatum* and *Oxytricha fallax*, which can be considered as ideal organisms for investigating water quality fluctuations and risk assessment of pesticides. Cell viability, cell morphology, cell behaviour and macronucleus tests were performed using to different concentrations of monocrotophos. The calculated LC_{50} value of monocrotophos against mortality curve for 3hrs exposure to *Paramecium caudatum* and *Oxytricha fallax* was 332.284±57.52ppm and 307.744±33.27ppm respectively. It was observed that the *Oxytricha fallax* was sensitive and *Paramecium caudatum* was highly responsive to monocrotophos. After a short period of exposure (20min to 30 min), there was an increase in the number of necrotic cells with typical features like blackening of cytoplasm, blebbing, leaking of internal contents and macronuclear changes leading to cell lysis. Changes in the contractile vacuole activity, size and shape of the organisms and macronucleus shape were also noticed. The present findings indicate a possible necrotic and ecogenotoxic effect of monocrotophos to *Paramecium caudatum* and *Oxytricha fallax* and such type of assays suggest the potential of above organism for ecotoxicological studies to certain pesticides.

Key words: *Paramecium caudatum, Oxytricha fallax,* monocrotophos, acute toxicity, macronuclear changes.

INTRODUCTION

The study of ciliate sensitivity to a wide number of toxic substances may provide a yardstick for identifying the intensity and potential for ecological damage caused by anthropogenic activities. For various technical reasons – small size, sensitivity, faster generation time, ubiquitous nature, and variety of trophic niches and size of genetic material - these models were best suited to screen toxicity effects of various pesticides (Morange, 2006). The attitude of scientists changing rapidly, first, for technical reasons; genome sequencing programmes have been extended to all organisms – including paramecia and this knowledge allows new experimental approaches to these organisms. In addition, ciliated protozoa are excellent unicellular animal models, as has been shown in two ciliates (*Tetrahymena* and *Paramecium*), with which humans share a higher degree of functional conservation than in other microbial models, and this is evidenced by better matches of these ciliate coding sequences to humans than other non-ciliated microbial model organisms (Narasimhan, 1999). Studies of the relationships between protozoa and physicochemical and operational parameters have revealed that the species structure of these communities is an indicator of water quality. The protozoa community is a complex assemblage of interacting organisms, often including species that are sensitive, resistant or intermediate in their tolerance to pollutants. Tests on the acute toxicity of pollutants on ciliates have revealed that these microorganisms are useful bioindicators for evaluating the toxicity of waters polluted by different pollutants (Madoni, 2011). In this context, the ciliate toxicity assay has become a valuable tool for detection of environmental disturbance and for assessment of the trophic state. In this context, the main purpose of this study is an attempt to evaluate the sensitivity of *Paramecium caudatum* and *Oxytricha fallax* to monocrotophos and to obtain a predictive tool for hazard and risk assessment in the water quality criteria.

MATERIALS AND METHODS

Test compound

The commercial grade sample of monocrotophos was supplied by Hyderabad chemical suppliers Ltd., Hyderabad. Monocrotophos (3-hydroxyl-N-methyl-cis-crotanamide dimethyl phosphate) ($C_7H_{14}NO_5P$), an organophosphorus compound, is a broad spectrum systemic insecticide and acaricide.

Experimental organisms

Protozoan ciliates *Paramecium caudatum* and

Oxytricha fallax were selected as test species. They were collected and isolated from freshwater pond within the vicinity of Osmania University campus, Hyderabad, India. The organisms were cultured in sterilized hay infusion medium in the laboratory at room temperature. Hay infusion medium has been widely used as a basic and most appropriate culture medium and it gives an environment nearest to their own habitat (Shiny *et al.,* 2005). Six grams of dried hay was boiled in one liter distilled water, cooled and filtered. Then, it was sterilized in an autoclave for 15 minutes at 15 pounds. Cooked ladies finger was supplemented to boost the bacterial multiplication and sterile precautions were taken in its use. For culturing the organisms, hay infusion medium was diluted with double distilled water in the ratio of 1:1 and was poured into different cavity blocks.

Acute toxicity studies

Stock solution and experimental concentrations of monocrotophos were prepared as recommended by APHA (2005). Stock solution of 10000ppm of monocrotophos was prepared diluting with distilled water. After preliminary range findings, the appropriate stock solution and the test concentrations were selected, prepared afresh and used for further studies. Acute toxicity tests were conducted 3hrs duration as suggested by Apostol (1973). In acute experiments 0.5 ml of pesticide solution was added to 4.5 ml of culture medium to achieve desired concentration of pesticide. 50 organisms were introduced in each cavity block. Triplicates were maintained for all concentrations. The cavity block, after adding pesticide was placed under binocular microscope and counting was done at intervals of 10 minutes during first one hour and thereafter 20 minutes interval during the next two hours. LC_{50} value was calculated against the mortality curve for 3 hrs. Controls devoid of pesticide, with same number of organisms were run simultaneously.

Macronucleus changes

Cytochemical studies were conducted to demonstrate the nuclear morphology of *Paramecium caudatum* and *Oxytricha fallax* on exposure to sub-lethal concentrations of monocrotophos. Nuclear staining was done by Methyl Green Pyronin-Y Method as suggested by Hussain (1984). 2% aqueous solution of pyronin was extracted with chloroform by shaking in a separating funnel until the chloroform layer became colorless. Methyl green extraction was also done in the same manner. For usage 12.5 ml of pyronin-y solution and 7.5 ml of methyl green was mixed with 30ml distilled water. For staining, the treated and control cells were air dried. Then the organisms fixed in 4% formaldehyde fixative were immersed in methyl green pyronin-y solution for six minutes. They were then rinsed in two changes of n-butyl alcohol for one minute each and then the slides were dehydrated quickly, cleared in xylene and mounted in DPX. The cytoplasm stains pink color and nucleus bluish green.

Statistical analysis

All the results were presented with suitable statistical interpretation such as test of significance, Mean and SD using origin 6.1software.

RESULTS AND DISCUSSION

Acute toxicity and behavioral studies

The objective of the acute toxicity study was to evaluate the sensitivity and survival capacity of freshwater ciliates *Paramecium caudatum* and *Oxytricha fallax* to monocrotophos. Immediate lethal concentration in which instant death of the organism occurred was determined; LC_{50} value and sub-lethal concentrations were worked out. Higher concentrations of monocrotophos increased cellular activity within a minute of exposure due to cell lyses. The calculated LC50 value of monocrotophos against mortality curve for 3hrs exposure to *Paramecium caudatum* and *Oxytricha fallax* was 332.284±57.52ppm and 307.744±33.27ppm respectively. At 150ppm concentration, cells became irritated within minutes of exposure and started swimming away. At moderate concentrations *Paramecium caudatum* showed vacuolization, formation of trichocysts and morphological deformities such as swollen body shape and shortened body length through anterior posterior axis. Abnormal behaviors such as restlessness, sudden and quick movements and swimming on the back at higher concentrations were also observed. Loss of movement coordination and orientation was observed at 50ppm. Rao (2004) reported LC_{50} value of monocrotophos and its analogs 2-butenoic acid-3-methyl ester and 2-butenoic acid-3-ethyl ester on euryhaline fish *Oreochromis mossambicus* at 96 hrs. Exposed fish exhibited abnormal behavior, which includes erratic swimming, loss in equilibrium and loss of mucus on gills. The dose-dependent effects of single-walled carbon nanotubes (SWNTs) on the ingestion and digestion of bacteria by *Tetrahymena thermophila*, was reported by Parnian *et al.,* (2008). Tetrahymena were able to internalize large quantities of SWNTs and then excrete SWNTs and undigested bacteria in aggregates. At high tube concentrations cell viability was also affected. The toxic effects of nanoparticles of ZnO

and CuO to particle-ingesting model organism protozoa *Tetrahymena thermophila* were evaluated by Mortimer *et al.,* (2010). Nano-ZnO was remarkably more toxic than nano-CuO. Toxic effect of CuO depended on particle size. The toxic effect of both, CuO and ZnO (nano) particles to protozoa was caused by their solubilised fraction. Abdul *et al.,* (2008) reported the ciliate, *Euplotes mutabilis*, isolated from industrial wastewater of tanneries showed tolerance against Cd^{2+} (22 μg ml^{-1}), Cr^{6+} (60 μg ml^{-1}), Pb^{2+} (75 μg ml^{-1}) and Cu^{2+} (22 μg ml^{-1}). The live protozoans could remove 97% of Pb^{2+} and 98% of Cr^{6+} from the medium, 96 h after inoculation of the medium containing 10 μg ml^{-1} of metal ions. When the ciliate was exposed to heavy metals at a larger scale it removed 86% of Pb^{2+} and 90% of Cr^{6+} from the medium. The metal uptake ability of *Euplotes mutabilis* was evidenced by its survival and normal growth in the culture medium. Nilsson (1999) reported that the Sodium orthovanadate at 0.1–5.0 mM affected cell proliferation of Tetrahymena in a dose-dependent manner. Endocytosis was affected in both a time- and dose-dependent manner; an increasing number of cells did not form vacuoles. Cell motility increased initially in 0.1 mM vanadate but decreased later as it did in 0.5–2.0 mM vanadate where the proportion of immobile cells increased with time. Cell divisions occurred at all concentrations but macronuclear elongation was disturbed and subsequent cytokinesis resulted in daughter cells containing the entire G_2 macronucleus, a large or small portion of it, or no nucleus at all. Moreover, odd cell shapes appeared with time. The size of the cell and nucleus increased but there was great variation with disturbed cytoplasm/nucleus ratios. The effects of indomethacin on the course of cell division, cyclin expression, the cortical microtubular system and on cytoskeleton-dependent processes (motility, phagocytosis) were investigated by Kovács and Pállinger (2003) in Tetrahymena. Indomethacin affected Tetrahymena in a number of ways: the structure of the cortical microtubular system became irregular and elongation of the macronucleus was noticed, which is a typical phenomenon of the normal course of mitosis. The cell growth rate, motility and phagocytotic activity were all considerably reduced. There are probably additional mechanisms responsible for the effect of indomethacin on the systems that control divisional morphogenesis, for microtubule-dependent processes and for the connection between nuclear and cortical alterations during the cell cycle. Golam *et al.,* (2005) reported that, the cell shape may depend on the cytoskeletal structures such as microtubules and actin filaments in *Paramecium bursaria*. The effects of heavy metal ions on cytoskeletal structures will disturb the shape of *Paramecium bursaria.* The correct size, shape and basic morphology of all ciliates depend on the correct structure and function of the cell membrane, which were being violated after adding this pesticide. The complete lysis of cells at a particular concentration of pesticide tested might be due to the natural response to adverse environment.

Table 1: Monocrotophos induced macronuclear changes (%) in *Paramecium caudatum* exposed for 72hrs and stained with Methyl Green Pyronin-Y Method.

Conc/ppm	100ppm	10ppm	1ppm	Control
Unevenly divided	13±1.94	11±2.82	7±2.54	____
Vacuolated	10±1.56	7±1.58	4±1.15	____
Fragmented	6±1.82	4±1.15	2±0.94	____
Rod Shaped	11±2.82	9±2.30	7±1.58	0.3±0.21
Others	12±1.56	9.2±1.483	4±1.15	0.2±0.32
Total abnormalities	51.6±2.95	40±1.88	24±2.78	0.5±0.53

Mean and SD values are significantly different from control groups at P<0.01. (n=5)

Table 2: Monocrotophos induced Nuclear changes (%) in *Oxytricha fallax* exposed for 72hrs and stained with Methyl Green Pyronin-Y Method.

Conc/ppm	100ppm	10ppm	1ppm	Control
Diffusion	12±1.56	8.8±1.48	5±1.41	_____
Disappeared	8.8±1.30	5±1.41	3±1	_____
Fragmented	9±1.22	7.2±1.30	4±1.15	_____
Rod Shaped	8.2±0.83	5±1.41	2±0.70	_____
Others	7.2±1.30	6.6±1.14	4.2±1.30	0.052±0.08
Total abnormalities	43.4±2.40	31.2±1.31	18.4±1.51	0.052±0.08

Mean and SD values are significantly different from control groups at P<0.01. (n=5)

Macronucleus changes

The macronuclear aberrations study on both treated and untreated *Paramecium caudatum* and *Oxytricha fallax* was performed to diagnose the morphological abnormalities in the nuclear shape, as a consequence of exposure to monocrotophos due to its cytogenetic action. Unevenly divided nucleus has been observed as the most common aberration and Rod shaped was the second common abnormality in Paramecium. The highest abnormalities (51.6±2.95) were recorded against 100ppm monocrotophos for 72 hrs. In concentrations of 100ppm, 10ppm and 1ppm, the percent abnormal forms recorded were 51.6±2.95, 40±1.88, and 24±2.78 respectively (Table 1). In Oxytricha complete diffusion of nucleus has been observed as the most common aberration and on exposure to 100ppm, 10ppm and 1ppm of monocrotophos, the percent aberrations observed were 43.4±2.40, 31.2±1.31 and 18.4±1.51 respectively (Table 2). The macronuclear aberrations were also observed in control cells in very less number because of abnormal growth and physiological status of a very few organisms. The macronucleus of ciliates is center of the entire series of metabolic activities and in its absence the animal soon dies. Cells showing nuclear aberrations are probably cell division failures and degenerative forms nearing to death. Formation of these abnormalities would represent a way to eliminate any amplified genetic material from the cell nucleus. This study indicated that higher concentration of monocrotophos has a cytogenetic potential. Kasturi bai and Dilli (1974) reported the toxicity of folidol on the morphology and nucleus of 5 fresh water ciliates namely *Spirostomum ambiguum major, Spirostomum amgiuum minor, Blepharisma intermedium, Blepharisma seshachari* and *Frontonia leucas.* All the test concentrations of folidol caused toxic effects on the nuclear apparatus and morphology bringing about changes such as reduction in its size, distortions, broken type, distorted nucleus and double macronuclei. Hussain *et al.,* (2008) found that when *Paramecium caudatum* was exposed to 280ppm concentration of carbofuran, it caused 47% abnormalities in macronucleus, which included deformities such as fragmentation, uneven division and vacuolization. Similarly, Amanchi (2010) and Amanchi and Hussain (2008) reported azadirachtin and delfin induced behaviour and nuclear changes in *Paramecium caudatum* and *Oxytricha fallax*. In conclusion, the present study gave us comprehensive understanding about the toxic effects of monocrotophos on *Paramecium caudatum and Oxytricha fallax* and their sensitivity and survival ability has made it as cheap, simple and idle candidates for the toxicity assessment of pesticide and pollution monitoring studies. Therefore, use of these eukaryotic microorganisms in ecotoxicological studies as potential whole cells would seem to be a reasonable and useful approach for humans.

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